Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Olotu A, Fegan G, Wambua J, et al. Four-year efficacy of RTS,S/AS01E and its interaction with malaria exposure. N Engl J Med 2013;368:1111-20. DOI: 10.1056/NEJMoa1207564

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Investigator and sponsor roles

The original study was sponsored by GlaxoSmithKline Biologicals, the vaccine developer and manufacturer, who monitored the trial and managed the database until 12 months of follow-up. The extension after 12 months was an investigator led study. This was sponsored by KEMRI-Wellcome Trust Collaborative Research Centre and funded partly by the PATH Malaria Vaccine Initiative and partly by the Wellcome Trust. GSK Biologicals employees reviewed and commented on the protocol and analysis plan, and were authors on the manuscript and therefore reviewed and approved the final submitted manuscript. A Malaria Vaccine Initiative employee reviewed and commented on the analysis plan, and is a contributing author on the manuscript. The Wellcome Trust had no role in protocol design, analysis, or publication.

The database and monitoring for the study extension after 12 months were managed by KEMRI-Wellcome Trust Collaborative Research Centre. All authors reviewed and approved the final manuscript presented. The first draft of the manuscript was written by Ally Olotu. All authors contributed to revisions of the manuscript, and approved the final manuscript. Revisions were coordinated by Ally Olotu. The data were subject to a confidentiality agreement between the GSK Biologicals, manufacturer of the RTS,S/AS01E and investigators, which established full access to the study data by the investigators and included an obligation to permit publication without excessive delay.

Study design

We originally conducted a double blind randomized controlled trial in Kilifi, Kenya and Korogwe, Tanzania. Kenya to evaluate the efficacy and safety of RTS,S/AS01$_E$ between March 2007 and November 2008[1]. The double-blind phase was completed in November 2008, after an average of 8 months follow-up post dose 3. During the single blind phase, only principal investigators were unblinded. Parents/guardians of participants, community health workers, laboratory staff, project manager, data manager and study clinicians remained blinded. The single blinded ended after an average follow-up of 12 month. Korogwe had no available infrastructure to continue with the follow-up and therefore the extension of follow-up beyond 12 months was carried in Kilifi, Kenya only. The original study was sponsored by GlaxoSmithKline Biologicals. Extended follow-up beyond 12 months was investigator-led, and sponsored by the KEMRI-Wellcome Trust Research Programme. The study is registered with ClinicalTrials.gov, number NCT00872963.

Location and site details

The original study was carried out in two sites; Kilifi Kenya and Korogwe, Tanzania[1]. In this manuscript, we present the data from Kilifi, Kenya only. The transmission intensity has previously been measured as 22-53 infective bites per year in Junju, Kilifi, but malaria transmission has been falling since 1999[2].

Original study supported free ITN distribution in the study area and there exist a successful ITN distribution program in Kenya. The first line antimalarial treatment is artemether/lumefantrine.

No insecticide spraying campaigns was reported in the study area during the entire follow-up period. The study area is rural and most of the population is subsistence farmers.

Study Participants: Screening

The participating children were aged 5-17 months old (inclusive) at the time of first vaccination, healthy, and resident in the study area. The details of exclusion and inclusion criteria of the original study are described elsewhere [1].

All subjects who were recruited in the original study were eligible for recruitment in the extension study. No recruitment outside the original cohort took place. Parents and/or guardians were informed of the objective of the extension and it was made clear that participation was voluntary.

Vaccines

Children were randomized to receive three doses of RTS,S/AS01$_E$, i.e. RTS,S with the proprietary Adjuvant System AS01E comprising liposomes, MPL (3-D-deacylated Monophosphoryl Lipid A) and QS21 (a triterpene glycoside purified from the bark of Quillaja saponaria) or 3 doses of Sanofi-Pasteur's human diploid cell rabies vaccine. The details of the vaccinees and vaccination procedure are described elsewhere [1]. No additional vaccination was conducted during extension study.

Randomization and Unblinding

Details of randomization have been published before[1]. Briefly The RTS,S/AS01E and rabies vaccines were in identical boxes labeled with treatment number. Randomization code was generated by a computer by the sponsor. Assignment of treatment number was on the basis of first come first serve. The study nurses who gave the vaccinations were unmasked to treatment, but the investigators, study participants, and parents or guardians of study participants were blinded to treatment assignment.

The double-blind of the study ended after an average of 8 months follow-up post dose 3. During the single-blind phase, only principal investigators AO and PB were unblinded allow the analysis of the data. However all participants, study clinicians in the community health workers in the field, project manager, data manager and laboratory staff remained blinded[3]. Parents were informed of the decision and the reason of not to unblind them before they were recruited into the extension study.

Assessment of Safety

The details of assessment of solicited, unsolicited adverse events have been described elsewhere [1]. In the extension study, surveillance of serious adverse event continued through health care system in place from original study. Details of all outpatient attendances and admissions to health facilities were reviewed by study clinicians to identify those meeting the criteria for Serious Adverse Event (SAE) reporting. In the case of a death which has occurred at home, supplementary information was sought by verbal autopsy technique. The verbal autopsy was

conducted according to previously published methods and detailed in the SOPs on file with the investigators [1].

Field workers were readily available to subject's families during the course of the trial. Field workers had access to study clinician for consultation by mobile phone 24 hours a day, 7 days a week and transport to the secondary heath facility was available 24 hours a day, 7 days a week if necessary. The two study health facilities Junju and Pingilikani Dispensaries were open during normal working hours and clinically qualified personnel were present at all times. All expenses including transport incurred by the parents/guardians of study participants for the purpose of obtaining a diagnosis of SAE as well as for clinical care related to acute conditions were borne by the study. Long-term care for chronic conditions unrelated to study procedures were delivered following local guidelines with no financial support from the study.

Surveillance of clinical malaria episodes

Surveillance of clinical malaria episodes was conducted through active and passive case detection method which started 2 weeks post dose 3. The details of the active and passive surveillance have been described before [1]. Briefly, active surveillance was conducted weekly and at each visit the axillary temperature of each subject was taken and if ≥ 37.5°C a blood slide and a rapid test (OptiMal®, Flow Incorporated, Oregon, USA) to determine malaria parasitaemia was taken. Treatment for episodes of malaria was with artemether-lumefantrine. Children requiring admission and too unwell to take oral medication were treated with intravenous quinine. If the parents/guardians reported that the child had fever but the axillary temperature was found to be < 37.5°C, the field worker revisited the child between 6 to 12 hours later to recheck the temperature.

Parents could bring their children for assessment between scheduled visits if they thought the child had developed fever, and the child was assessed in the same way. Fieldworkers were stationed in the study villages, and so were readily accessible to the parents. Passive case detection was also established in local dispensaries providing care to the population.

Malaria treatment was indicated by rapid test result. Blood slides for parasitaemia were not red in real time to guide case management but their results were necessary for endpoint determination. Criteria for severe malaria were derived from the WHO definition as described before[1].

Cross sectional survey and laboratory Methods

We obtained blood samples to study antibodies to *Plasmodium falciparum* circumsporozoite repeat region (anti-CS antibodies) and/or asymptomatic parasitaemia; before vaccination at 1 month, ~8 months (range 4–10 months), 12 months, ~15 months (range 12-18 months), ~25 months (range 21-27 months), ~38 months (range 34-40 months) an~49 months (range 45-51 months) post dose 3. Anti-CS antibodies were measured by ELISA at the Centre for Vaccinology (CEVAC; Ghent, Belgium) as described previously [1] and reported in EU/mL. Plates were adsorbed with the recombinant antigen R32LR that contains the sequence [NVDP(NANP)15]2LR.

Thick and thin films for parasite density readings were made in the laboratories in Kilifi district hospital and stained with giemsa. All laboratory staff was blinded and had no subject's detail with exception of their study number and type of visit. The details of slide reading and calculation have been described elsewhere [1].

Malaria exposure

We developed malaria exposure index based on local prevalence of malaria infection around each child as described in details elsewhere [4]. The justification for use of such a measure is that a) the prevalence of malaria infection in community is a widely used as a measure of regional transmission intensity[5] and b) the prevalence of malaria infection is highly structured on fine spatial scales within regions [6].

In brief, data from study cohort and another cohort of individuals under active surveillance within the same study area were used. The data were prospectively collected by active and passive surveillance and annual cross-sectional bleed during the follow-up of extension study. We computed distances (in Kilometers) from each individual to all others in the combined cohort. The weighted local prevalence was calculated as distance-weighted proportions of malaria infected children within an area of 1 km radius and 6 month time interval. The nearest infections were given more weight than the distant infections in determination of exposure index.

Exposure indices were validated using malaria infection of an individual index case. An index child's malaria status was not used to assess exposure index. Receiver operating characteristic curves were used to determine the discriminatory power of exposure index. The ROC curve for the exposure index in the study area was 0.71 (95%CI: 0.69-0.73). Children were categorized into either high or low malaria exposure if their exposure indices were above or below the cohort median during the respective follow-up period.

Statistical analysis

Missing data

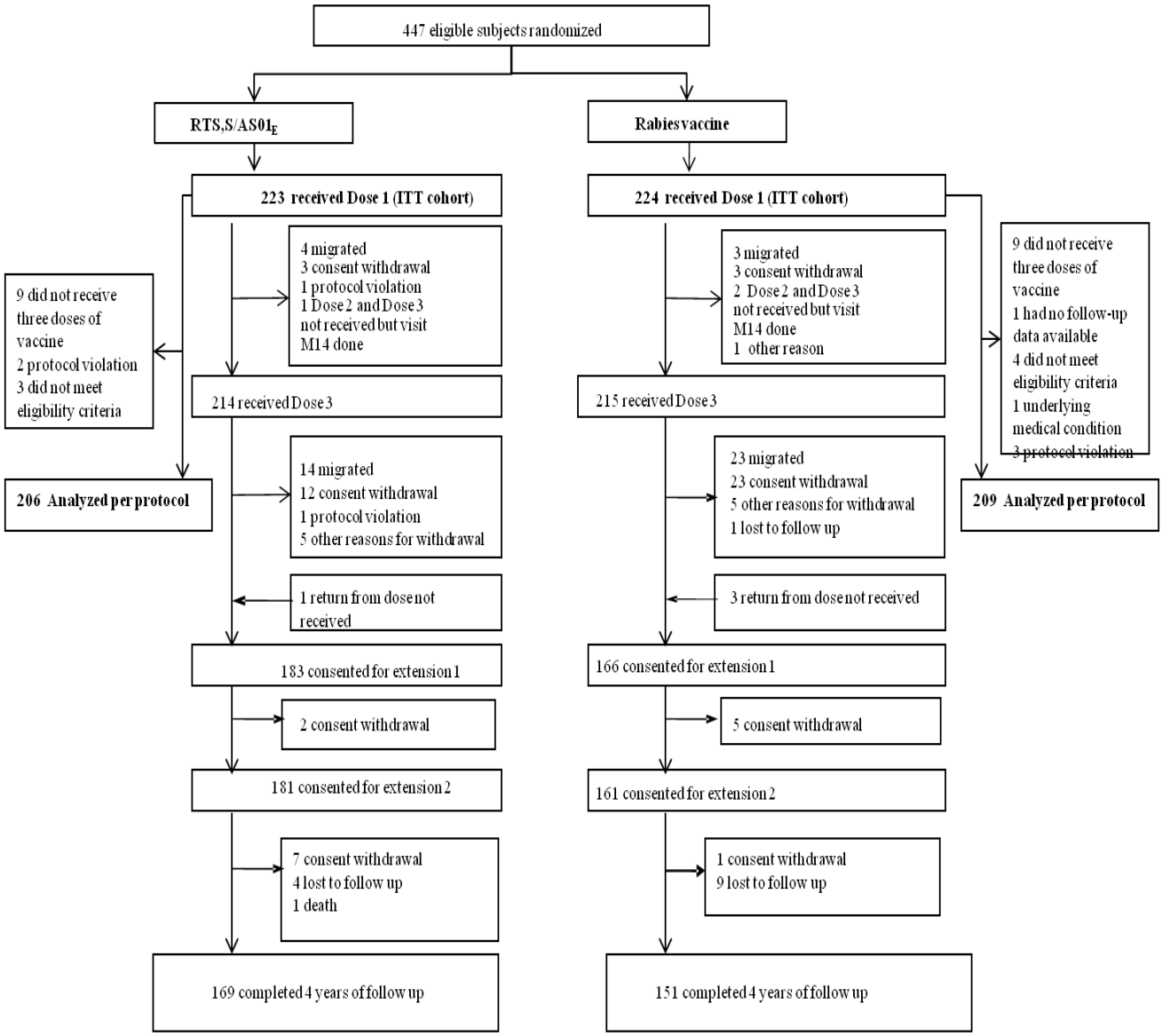
All available data were used for the relevant analyses (i.e. children were not removed entirely from analysis on the basis of incomplete data). We did not perform imputation of missing data or carry forward the last observation in place of missing data. In the analysis of multiple episodes by Negative binomial regression, time at risk was for each individual was calculated based on the time spent in the study, and the time at risk was used as the "offset" variable to account for loss to follow up. Twenty eight days was deducted from time at risk after each episode of clinical malaria to account for the effect of anti-malarial treatment. We did not adjust for brief time the participants spend away from the study area, since this was difficult to establish accurately.

Subgroup analyses

We explored for the existence of interaction between vaccination and malaria exposure index and conducted subgroup analyses on vaccine efficacy against multiple episodes meeting primary endpoint in children with high and low malaria exposure. We also performed stratified analysis of efficacy against multiple clinical malaria episodes by year of follow-up. These analyses were informed by the presence of significant interaction between vaccination and concerned subgroup characteristics.

Supplementary figures and Tables

Figure S1. Consort diagram



"Other" includes children missing vaccinations because of hospital admission, with contraindications to further vaccination, medical conditions not permitted by the protocol, and with no concomitant vaccination documentation.

Figure S2. Comparison of Negative binomial and Poisson distribution fit for the our data (According to protocol cohort)

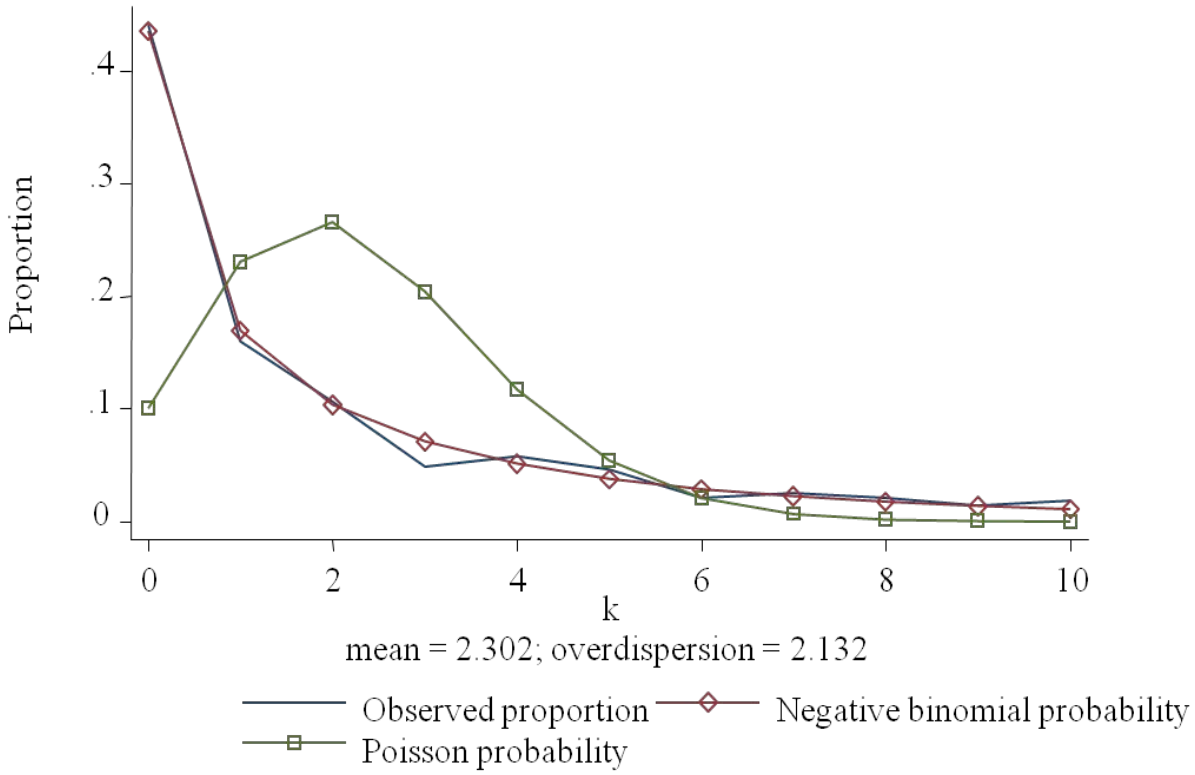


Table S1. Interaction analysis between vaccination, malaria exposure and time of follow-up (According protocol cohort)

	Negative binomial regression			**Andersen and Gill Cox regression**		
	RTS,S/AS01E N=209	Rabies N=206		RTS,S/AS01E N=209	Rabies N=206	
Covariate	IRR	95% Confidence interval	P value	HR	95% Confidence interval	P value
$RTS,S/AS01_E$	0.37	0.22-0.61	<0.001	0.23	0.12-0.45	<0.001
EI	4.67	2.18-10.03	<0.001	3.92	2.15-7.12	<0.001
$RTS,S/AS01_E$*EI	2.48	1.18-5.21	0.02	5.17	1.98-13.47	0.001
Area1	1	-	-	1	-	-
Area2	0.78	0.44-1.35	0.37	0.80	0.47-1.37	0.42
Area3	0.49	0.34-0.73	<0.001	0.56	0.38-0.84	0.005
Area4	0.45	0.29-0.69	<0.001	0.53	0.35-0.82	0.004
Bed net	0.82	0.66-1.02	0.08	0.77	0.62-0.95	0.02
Distance to dispensary	0.84	0.75-0.95	0.005	0.88	0.78-1.00	0.05
Age (months)	0.99	0.96-1.03	0.67	1.01	0.97-1.04	0.73
$RTS,S/AS01_E$* year 1	1	-	-			
$RTS,S/AS01_E$*year 2	1.37	0.83-2.27	0.22			
$RTS,S/AS01_E$* year 3	1.39	0.86-2.26	0.17	$1.28^{\S}$	1.08-1.51	0.004
$RTS,S/AS01_E$* year 4	1.65	1.03-2.63	0.04			
EI*year 1	1					
EI*year 2	0.62	0.28-1.34	0.22			
EI*year 3	0.35	0.15-0.82	0.02	$0.71^{€}$	0.51-0.98	0.04
EI*year 4	0.35	0.16-0.79	0.01			
Year 1	1	-	-	NA	-	-
Year 2	0.96	0.62-1.49	0.85	NA	-	-
Year 3	1.41	0.87-2.27	0.16	NA	-	-
Year 4	1.56	1.02-2.37	0.04	NA	-	-

§: interaction between vaccination and time as continuous variable, €: interaction between exposure index and time as continuous variable, EI: Malaria exposure index,; IRR: Incidence rate ration; The models with three-way interactions (vaccination, year and exposure) didn't fit the data well; N=Number of children

Table S2. Stratified adjusted vaccine efficacy against all episodes by malaria exposure and year of follow-up using NB regression (According to protocol cohort)

	Total cohort RTS,S/AS01E (N)=209 Rabies (N)=206			Low exposure group RTS,S/AS01E (N)=93 Rabies (N)=100			High exposure group RTS,S/AS01E (N)=109 Rabies (N)=103		
	VE (%)	95% CI	P value	VE(%)	95% CI	P value	VE(%)	95% CI	P
All years	23.5	-0.7 to 41.9	0.056	45.1	11.3 to 66.0	0.014	15.9	-11.0 to 36.4	0.221
Year 1	46.2	21.2 to 63.4	0.001	58.2	7.2 to 81.3	0.032	40.1	9.9 to 60.4	0.014
Year 2	24.7	-19.1 to 52.3	0.225	39.2	-38.2 to 72.9	0.237	30.6	-1.1 to 53.3	0.058
Year 3	22.0	-17.0 to 48.0	0.23	62.3	24.0 to 82.4	0.006	-4.0	-61.2 to 33.0	0.862
Year 4	-1.2	-46.8 to 31.2	0.95	41.5	-7.5 to 69.4	0.085	-29.0	-98.2 to 14.8	0.223

VE: Adjusted vaccine efficacy estimates, CI: 95% Confidence Interval, NB: Negative Binomial, (N) Number of children

Table S3 Asymptomatic parasitaemia at cross-sectional bleed in the per-protocol cohort

Mean month (range)	**RTS,S/AS01E Vaccine**		**Rabies Vaccine**		**Efficacy (95% CI)**	**P Value**
	Participants Tested (N)	Participants with Positive Slides (N (%))	Participants Tested (N)	Participants with Positive Slides (N (%))		
8 (4-10)	193	2 (1)	184	8 (4)	76.9 (–17.9 to 97.6)	0.06
12	185	9 (5)	175	24 (14)	67.8 (25.4 to 87.2)	0.005
15(12-18)	167	2 (1)	146	9 (6)	81.5 (8.3 to 98.1)	0.03
25 (21-27)	161	6 (4)	139	10 (7)	50.1 (–56.8 to 85.5)	0.21
38 (34-40)	154	14 (9)	139	28 (20)	60.4 (17.5 to 81.6)	0.008
49 (45-51)	148	11 (7)	136	7 (5)	–47.9 (49.5 to –27.5)	0.47

Reference

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